Mechanism and effects of silencing green peach aphid genes via RNA interference *

V BILGI†, J FOSU-NYARKO & M G K JONES

Plant Biotechnology Research Group, WA State Agricultural Biotechnology Centre, School of Biological Sciences & Biotechnology, Murdoch University, Murdoch, WA 6150, Australia.

† Corresponding author ✉ vineetabilgi@gmail.com

When chemicals are used to control insect pests, the potential for resistant phenotypes to develop is well documented. Genetically modified plants expressing a bacterial protein (Bt) are now widely deployed, but are only effective against chewing insects. There is now an opportunity to exploit the highly conserved natural cellular process of RNA interference (RNAi) to silence specific genes in sucking insects such as aphids. RNAi can be triggered when double-stranded RNA (dsRNA) is introduced into a cell where a series of cellular processes lead to unwinding of the RNA and binding to a complex which degrades a target mRNA, leading to loss-of-function of its protein.

The aim of this research is to investigate the mechanism and effects of silencing essential genes of the green peach aphid (*Myzus persicae*). To identify target genes for the project, we will employ comparative genomics and bioinformatics tools to identify and characterise green peach aphid orthologues of genes essential for development using the expressed sequence tags of green peach aphid, the genomic data available for the related pea aphid, *Acyrthosiphon pisum* as well as genomic resources of the best-annotated multicellular organism to date, *Caenorhabditis elegans*. Selected genes would be characterised *in silico* using, for example, the functional genomic resources that details RNAi phenotypes of almost all known genes of the free-living nematode, *C. elegans*. In addition, genes encoding proteins secreted from the salivary glands (the secretome) would be characterised using RNAi by investigating their effects on the viability and reproduction of aphids when they are silenced. Currently, we have amplified and sequenced six target genes and used these to establish an *in vitro* RNAi system *via* dsRNA feeding of nymphs with artificial diet sachets containing sucrose and dsRNA corresponding to the target genes. These genes, involved in proton translocation, locomotion, olfaction and osmoregulation, share up to 90% nucleotide sequence homology with the pea aphid and have no sequence homologies with the human, mouse, *Arabidopsis* and tobacco genomes.

The mechanism of RNA silencing is not well-understood in insects. To study in detail how small RNAs are processed by insects, we will use dsRNA and small RNAs of different conformations and compositions to elicit gene silencing, and to investigate the lengths and types of sequences in constructs that produce the best RNAi response and effects on green peach aphid via *in vitro* feeding and through transgenic plants. After feeding on dsRNA, gene knockdown in nymphs will be assessed by quantitative polymerase chain reactions (qPCR) to measure transcript abundance in primary target aphids as well as in their progeny. Effects of silencing essential genes will be assessed by monitoring survival and fecundity of aphids on a host plant after feeding on dsRNA for 24 hours. This monitoring will be done at 24 hourly intervals for 16 days and the results compared with nymphs treated without dsRNA.

To evaluate the possibility of using RNAi as a control strategy for sucking insect pests, green peach aphid will be reared on transgenic plants modified to produce dsRNA corresponding to candidate RNAi genes which when silenced limit aphid reproduction and affect survival. This proof-of-concept will use model host plants such as *Arabidopsis thaliana* and *Nicotiana tabacum*. The results of this research will provide target genes that effectively disrupt development, reproduction and survival of green peach aphid, and lay a foundation for RNAi-mediated control of not only green peach aphid but many of the world's damaging aphid and insect pests.

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